American Journal of Medical Genetics 69:98–101 (1997)

Deletion of the Entire *NF1* Gene Causing Distinct Manifestations in a Family

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We identified a father and son with neurofibromatosis type 1 (NF1) due to a deletion of the entire *NF1* gene detected by fluorescence in situ hybridization (FISH). As is the case for others reported to have such large deletions, father and son had severe NF1, including a large number of cutaneous neurofibromas, facial anomalies, large hands, feet, and head, and developmental impairment. They were discordant in that seizures and plexiform neurofibromas occurred only in the propositus. Large NF1 deletions can be compatible with familial transmission and appear to be associated with a distinct phenotype. Am. J. Med. Genet. 69:98–101, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: NF1; FISH; *NF1* gene deletion; familial deletion; neurofibromas; facial anomalies; learning disability

INTRODUCTION

Neurofibromatosis type 1 (NF1) is an autosomal-dominant disorder caused by mutations in the *NF1* gene which spans 335 kb at 17q11.2 [Wallace et al., 1990; Viskochil et al., 1990; Cawthon et al., 1990; Marchuk et al., 1991; Li et al., 1995]. Phenotypic signs include café-au-lait macules, neurofibromas, skeletal dysplasia, developmental delay, and, in some cases, abnormal facial appearance, and learning disability or mental retardation. The disorder is characterized by a wide range of variability of expression within and between families [Huson et al., 1989; Viskochil and Carey, 1992; Easton et al., 1993]. Genetic analysis of the *NF1* gene has indicated a wide diversity of mutation types, and genotype-phenotype correlations have been difficult to establish [Upadhyaya et al., 1992, 1994].

The recent identification of large *NF1* deletions in 11 patients with severe manifestations [Kayes et al., 1994; Wu et al., 1995; Leppig et al., 1996] suggests that deletion of the entire *NF1* gene, perhaps including adjacent genes, may be associated with a distinct phenotype characterized by early onset of cutaneous neurofibromas, facial anomalies, and learning disability or mental retardation, in addition to more common findings of NF1. Two of these studies [Wu et al., 1995; Leppig et al., 1996] also demonstrated that fluorescence in situ hybridization (FISH) provides simple, rapid, and unambiguous detection of large *NF1* deletions. All *NF1* gene deletions reported to date have been sporadic cases. We describe a father and son with deletion of the entire *NF1* gene who share a severe phenotype.

CLINICAL REPORTS

The propositus (case 95-629) is a 15-year-old African American male first evaluated for NF1 at age 3 years. He was the 2.8-kg product of an uncomplicated pregnancy, born spontaneously at 36 weeks of gestation. NF1 is present in the patient's father and epilepsy in his mother. There is no other documented family history of NF1.

Examination at age 3 years showed café-au-lait spots, skin-fold freckles, and Lisch nodules, but no neurofibromata. Cutaneous neurofibromas appeared by age 5 years. There are two plexiform neurofibromas. He has complex partial seizures with a right temporal focus, which developed at age 4 years. No structural lesions have been seen in the central nervous system by MRI. He is delayed in cognitive and language function and requires special classes. Examination at age 15 years shows innumerable cutaneous and subcutaneous neurofibromas (Fig. 1A). Facial anomalies include broad nasal bridge and downslanting palpebral fissures (Fig. 1C). Hands and feet are disproportionately large (Fig. 1E), and head circumference is at the 98th centile.

The father of the propositus (case 95-940) is now 45

Contract grant sponsor: NIH/NICHD; Contract grant number: 5P30HD18655-13.

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Received 6 June 1996; Accepted 2 October 1996

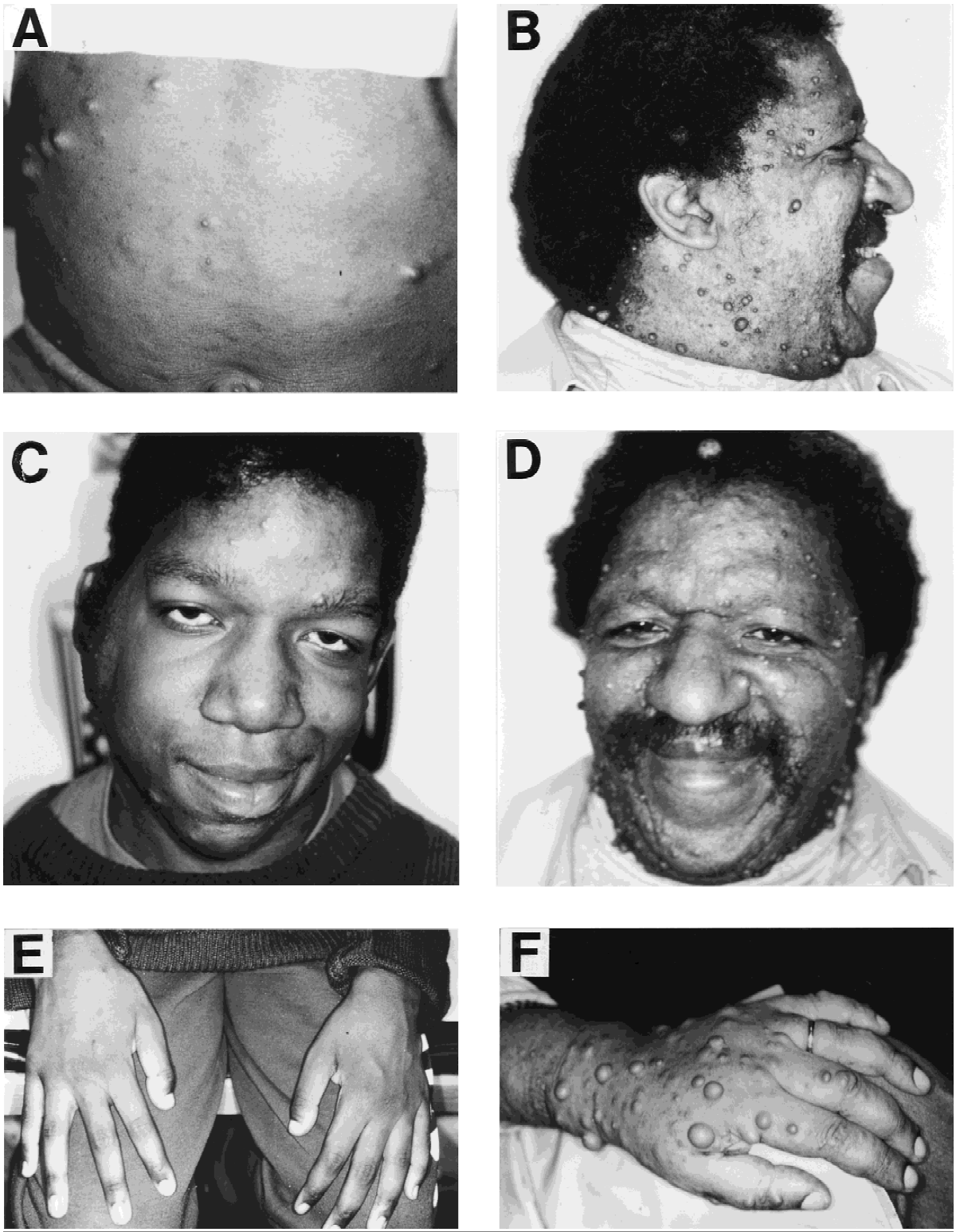

Fig. 1. Manifestations of NF1 in the propositus (95-629, left) and the father (95-940, right). Innumerable cutaneous and subcutaneous neurofibromas appeared on the abdomen of the propositus **(A)** and on his father's face **(B)**. Facial appearance of the propositus **(C)** and his father **(D)**. Large size of hands of the propositus **(E)** and his father **(F)**.

years old. He has had migraines, mitral valve prolapse, and hypertension. He is a high school graduate and is employed as a maintenance worker. He has manifestations similar to those of his son, including café-au-lait spots, generalized freckling, Lisch nodules, and innumerable cutaneous and subcutaneous neurofibromas (Fig. 1B), but no plexiform neurofibromas. Facial anomalies include broad nasal bridge and downslanting palpebral fissures (Fig. 1D), and he has large hands, feet, and head (Fig. 1F). Figure 1 shows the comparison of these anomalies in the propositus and his father.

MOLECULAR CYTOGENETICS STUDIES

FISH studies with a set of intragenic cosmids (cFF13, cFB5D, and cP5) and YACs (yA43A9, yD8F4, and yA113D7) that span the entire NF1 gene were performed on cultured leukocytes from the propositus, and from the father, mother, and brother of the propositus. Probe DNA from the cosmids and the entire YAC clones was isolated by routine molecular techniques. Purified YAC clones were then amplified by Alu-PCR with primer AGK34 for selective amplification of human sequences [Baldini et al., 1992]. All NF1 probes, including cosmid, Alu-PCR products of YAC, and entire YAC clones, were labeled with biotin-11-dUTP by nick-translation, while reference probes were labeled with digoxigenin-16-dATP. FISH methods for denaturation, hybridization, high stringency washing, and detection were the same as described in detail previously [Wu et al., 1995, for single-color FISH; Wu et al., 1997, for dual-color FISH]. Thirty metaphase cells with DAPI counterstaining were analyzed and photographed by the Cytovision, version 2.24 (Applied Imaging, Pittsburgh, PA). The three intragenic cosmid probes were used first, which correspond with the 5′, central, and 3′ regions of the *NF1* gene, respectively. Deletions were confirmed with the YAC contig, which consists of three overlapping clones that span the entire *NF1* gene and extend into approximately 700 kb of the surrounding region [Marchuck et al., 1992]. The results showed that both the propositus and father have a large deletion, lacking hybridization to probes cFF13, cFB5D, cP5, yA43A9, yD8F4, and yA113D7 on one chromosome 17, indicating that this deletion encompasses the entire *NF1* gene. To further confirm and estimate minimum size of the deletion, the entire YAC contig was labeled and hybridized because the initial YAC probes were Alu-PCR products, which cannot show for certain that the complete length of the DNA encompassed in this contig was deleted. As expected, the whole YAC contig was deleted; thus the deletion size is at least 700 kb. Figure 2 shows the FISH results. The mother and brother of the propositus do not have this deletion. The deletion was not visible cytogenetically at a resolution of 550 bands.

DISCUSSION

The wide diversity of mutation types and wide range of variable expression of neurofibromatosis 1 have made it difficult to establish genotype-phenotype correlations [Huson et al., 1989; Huson, 1994; Viskochil and Carey, 1992; Upadhyaya et al., 1992, 1994]. Nev-

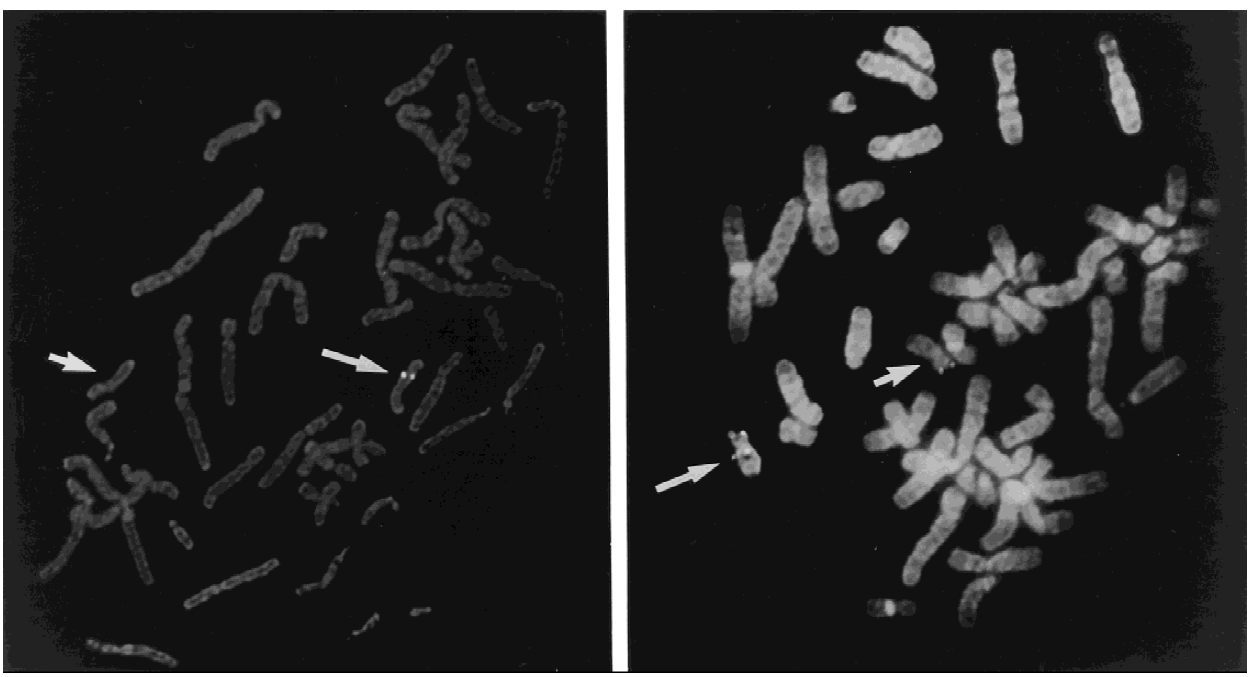

Fig. 2. Examples of FISH results from the propositus (95-629, left) and the father (95-940, right). **Left:** Single-color detection with one-probe hybridization for the propositus. Only one chromosome 17 hybridized with YAC clone yA43A9 (in this black and white figure long arrow points to the normal 17, and short arrow to the deleted 17, which has no hybridization signal at 17q11.2). **Right:** Dual-color detection with two-probe hybridization for the father. The deleted chromosome 17 hybridized with reference probe P53 only (red, at 17p13.1, indicated by short arrow in this black and white figure), while the normal 17 hybridized with both cosmid clone cFB5D (green, at 17q11.2) and reference probe P53 (red, at 17p13), indicated by long arrow in this black and white figure.

ertheless, deletion of the entire *NF1* gene appears to be associated with a distinct phenotype that includes early onset of a large number of cutaneous neurofibromas, minor facial anomalies, and developmental delay [Kayes et al., 1994; Wu et al., 1995; Leppig et al., 1996]. Hitherto, all published cases have been sporadic. We report here a father and son with a large deletion; one other example of parent-to-child transmission of an *NF1* deletion was reported in abstract form [Cnossen et al., 1995].

Detection of an identical mutation in a parent and child provides an opportunity for analysis of clinical variability in NF1. Our propositus and his father have similar manifestations, including café-au-lait spots, freckling, Lisch nodules, innumerable cutaneous and subcutaneous neurofibromas, facial anomalies, large hands, feet, and head, and cognitive impairment. It is possible that some of the facial characteristics and the large hands and feet are familial, although the phenotype is consistent with that of the other 15 reported sporadic cases [Kayes et al., 1994, with 5 cases; Wu et al., 1995 and unpublished data, with 8 cases; Leppig et al., 1996, with 2 cases]. The extent to which this phenotype is unique to patients with NF1 and the large deletion will require a larger study of NF1 patients selected without bias for specific clinical characteristics.

Two symptoms occur in the propositus but not his father. First, only the propositus has seizures. It is unclear whether these are due to his NF1, or whether they are related to the history of epilepsy in his mother. Korf et al. [1993] found only 22 individuals with NF1 and seizures in a clinic population of 359 patients, suggesting that seizures are relatively uncommon in patients with NF1, and when they occur usually have a natural history similar to that of seizures in the general population. The second discordance is that the propositus has two large plexiform neurofibromas, whereas his father has none. Easton et al. [1993] studied 175 individuals in 48 NF1 families and found that plexiform neurofibromas did not show significant familial clustering, in contrast with number of café-au-lait spots or neurofibromas. It is possible that the occurrence of a plexiform neurofibroma represents a chance event in early development, regardless of the specific *NF1* mutation.

Fluorescence in situ hybridization provides a simple and sensitive approach to the detection of large *NF1* deletions [Wu et al., 1995; Leppig et al., 1996]. Although most cases reported so far have been sporadic, familial recurrence is possible. The full range of the phenotype associated with deletion remains to be explored, as more NF1 patients are studied by this approach.

ACKNOWLEDGMENTS

We thank Dr. Francis Collins and Dr. Douglas Marchuk for providing cosmid probes and YAC contigs, and we thank Benning Cao and Cindy Tellgmann for their assistance. This work was supported in part by NIH/NICHD grant 5P30HD18655-13.

REFERENCES

Baldini A, Ross M, Nizetic D, Vatcheva R, Lindsay EA, Lehrach H, Siniscalco M (1992): Chromosomal assignment of human YAC clones by fluorescence *in situ* hybridization: Use of single-yeast-colony PCR and multiple labeling. Genomics 14:181–184.

Cawthon RM, Weiss R, Xu G, Viskochil D, Culver M, Stevens J, Robertson M, Dunn D, Gesteland R, O'Connell P, White R (1990): A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutation. Cell 62:193–201.

Cnossen MH, van de Est MN, de Goede-Bolder A, Breuning MH, van Asperen CJ, Breslau-Siderius EJ, Halley DJJ, van den Ouweland AMW, Niermeiier MF (1995): Deletions spanning the neurofibromatosis type 1 gene: On the verge of genotype-phenotype correlations in NF1? Am J Hum Genet [Suppl] 57:237.

Easton DF, Ponder MA, Huson SM, Ponder BAJ (1993): An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): Evidence for modifying genes. Am J Hum Genet 53:305–313.

Huson SM, Campston DAS, Harper PS (1989): A genetic study of von Recklinghausen neurofibromatosis in southeast Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 2:704–711.

Huson SM (1994): Neurofibromatosis 1: a linical and genetic overview. In Huson SM, Hughes RAC (eds): "The Neurofibromatoses: A pathogenetic and Clinical Overview." London: Chapman and Hall Medical, pp 204–232.

Kayes LM, Burke W, Riccardi VM, Bennett R, Ehrlich P, Rubenstein A, Stephens K (1994): Deletion spanning the neurofibromatosis I gene: Identification and phenotype of five patients. Am J Hum Genet 54:424–436.

Korf BR, Carrazana E, Holms GL (1993): Patterns of seizures observed in association with nerofibromatosis 1. Epilepsia 34:616–620.

Leppig KA, Viskochil D, Neil S, Rubenstein A, Johnson VP, Zhu XL, Brothman AR, Stephens KG (1996): The detection of contiguous gene deletions at the neurofibromatosis 1 locus with fluorescence in situ hybridization. Cytogenet Cell Genet 72:95–98.

Li Y, O'Connell P, Breidenbach HH, Cawthon RM, Stevens J, Xu G, Neil S, Robertson M, White R, Viskochil D (1995): Genomic organization of the neurofibromatosis 1 gene. Genomics 25:9–18.

Marchuk DA, Saulino AM, Tavakkol R, Swaroop M, Wallace MR, Andersen LB, Mitchell AL, Gutmann DH, Boguski M, Collins FS (1991): cDNA cloning of the type 1 neurofibromatosis gene: Complete sequence of the *NF1* gene product. Genomics 11:931–940.

Marchuk DA, Tavakkol RM, Wallance MR, Brownstein BH, Taillon-Miller P, Fong C-T, Legius E, Andersen LB, Glover TW, Collins FS (1992): A yeast artificial chromosome contig encompassing the type 1 neurofibromatosis gene. Genomics 13:672–680.

Upadhyaya M, Shen M, Cherryson A, Farnham J, Maynard J, Huson SM, Harper PS (1992): Analysis of mutations at the neurofibromatosis (*NF1*) locus. Hum Mol Genet 1:735–740.

Upadhyaya M, Shaw DJ, Harper PS (1994): Molecular basis of neurofibromatosis type 1 (*NF1*): Mutation analysis and polymorphisms in the *NF1* gene. Hum Mutat 4:83–101.

Viskochil D, Carey JC (1992): Nosological considerations of the neurofibromatoses. J Dermatol (Tokyo) 19:873–880.

Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolef RK, Culver M, Carey JC, Copeland NG, Jenkins NA, White R, O'Connell P (1990): Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell 62:187–192.

Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, Fountain JW, Brereton A, Nicholson J, Mitchell AL, Brownstein B, Collins FS (1990): Type 1 neurofibromatosis gene: Identification of a large transcript disrupted in three NF1 patients. Science 249:181–186.

Wu BL, Austin MA, Schneider GH, Boles RG, Korf BR (1995): Deletion of the entire *NF1* gene detected by FISH: Four deletion patients associated with severe manifestations. Am J Med Genet 59:528–535.

Wu BL, Boles RG, Yaari H, Weremowicz S, Schneider GH, Korf BR (1997): Somatic mosaicism for deletion of the entire *NF1* gene identified by FISH. Hum Genet, in press.